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Ndufs4 related Leigh syndrome: A case report and review of the literature

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ABSTRACT

The genetic causes of Leigh syndrome are heterogeneous, with a poor correlation between the phenotype and genotype. Here, we present a patient with an *NDUFS4* mutation to expand the clinical and biochemical spectrum of the disease. A combined defect in the CoQ, PDH and RCC activities in our patient was due to an inappropriate assembly of the RCC complex I (CI), which was confirmed using Blue-Native polyacrylamide gel electrophoresis (BN-PAGE) analysis. Targeted exome sequencing analysis allowed for the genetic diagnosis of this patient. We reviewed 198 patients with 24 different genetic defects causing RCC I deficiency and compared them to 22 NDUFS4 patients. We concluded that NDUFS4-related Leigh syndrome is invariably linked to an early onset severe phenotype that results in early death. Some data, including the clinical phenotype, neuroimaging and biochemical findings, can guide the genetic study in patients with RCC I deficiency.

1. Introduction

Leigh syndrome is a progressive neurodegenerative condition of childhood characterized by lesions of the basal ganglia, thalamus, brainstem, and less frequently, the cerebellum. The symptoms of Leigh syndrome are highly variable, but usually include psychomotor arrest or regression, hypotonia, dystonia, seizures, ocular movements, respiratory failure and vomiting. Biochemically, elevated lactate levels in the blood and cerebral spinal fluid are frequently encountered (Anderson et al., 2008). Recently, reversible causes of Leigh syndrome were described to involve a thiamine transporter type 2 deficiency (Ortigoza-Escobar et al., 2014; Lake et al., 2015). Therefore, an analysis of thiamine derivatives in the CSF and early treatment with thiamine and biotin are recommended (Ortigoza-Escobar et al., 2016).

RCC I (CI- NADH-ubiquinone reductase) deficiency is the most frequently observed abnormality and accounts for ~30% of the cases of Leigh syndrome (Fassone and Rahman, 2012; Hoefs et al., 2012; Pagniez-Mammeri et al, 2012; Pagniez-Mammeri et al., 2009). CI is the largest multimeric enzyme of the mitochondrial RCC and has been shown to oxidize NADH, transfer electrons to CoQ and pump protons across the mitochondrial membrane (Scheffler, 2015; Mimaki et al., 2012; Antonicka et al., 2003).

The aim of this report was to describe a Moroccan infant with fatal early Leigh syndrome and a combination of PDH, RCC and CoQ deficiencies in muscle tissue, who was identified to have a mutation in the *NDUFS4* gene using massive parallel sequencing (MPS). Blue-Native polyacrylamide gel electrophoresis (BN-PAGE) revealed a complete absence of the fully assembled RCC I in the muscle tissue, thereby confirming the crucial role of *NDUFS4* in the assembly of functional RCC I (Anderson et al., 2008; Assereto et al., 2014; Budde et al., 2003; Hinttala et al., 2005, Leshinsky-Silver et al., 2009, Lombardo et al., 2014; Papa et al., 2001, Petruzzella et al., 2005). To better characterize the phenotype of patients with NDUFS4-related Leigh syndrome, we compared

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their phenotype to the phenotype of 198 patients with RCC 1 deficiency. Our results confirm that children with *NDUFS4* mutations show a homogeneous early onset and severe, lethal course of the disease in contrast to the broad clinical spectrum described in RCC 1 defects.

2. Methods

Blood samples, a muscle biopsy and fibroblasts from the patient were collected with the approval of the Institutional Review Board at Hospital Sant Joan de Déu.

The PDHc activity was determined in cultured fibroblasts and muscle tissue as previously reported (Guitart et al., 2009). The substrate oxidation rates were analysed in fibroblasts by measuring $^{14}CO_2$ production from [^{14}C]-pyruvate and [^{14}C]-glutamate (Willems et al., 1978). The total CoQ concentration was determined using HPLC with electrochemical detection (Montero et al., 2005).

We performed BN-PAGE to isolate intact protein complexes from the skeletal muscle. The assembly of the five oxidative phosphorylation complexes was examined using two-dimension blue native/SDS-PAGE. The gels were blotted and incubated with five antibodies specific to each mitochondrial complex.

Pre-mortem open biopsies were taken and muscle specimens were stained using standard procedures.

Total DNA was extracted from blood samples using the MagnaPure system (Roche Applied Science, IN, USA). Genetic analysis of nuclear DNA-encoded genes involved in mitochondrial disorders was achieved through targeted exome sequencing using the TruSight One Sequencing Panel (Illumina) as previously described (Vega et al., 2016a, 2016b).

3. Results

3.1. Case report

The patient was a female born after spontaneous vaginal delivery at 40-weeks of gestation with a birth weight of 2860 g and head circumference of 34 cm. Her Apgar scores were 9 and 10 at 1 and 5 min, respectively. Her prenatal history was unremarkable, except for pyelectasis, which resolved spontaneously. Her Moroccan parents were consanguineous (first cousins).

She presented at 37 days of age with paroxysmal abnormal ocular movements consisting of conjugate down-gaze deviation, convergent strabismus and horizontal nystagmus. Her neurological examination was otherwise normal, and a cranial ultrasound disclosed no abnormalities. At 2 months of age, she was admitted to the hospital with vomiting, lethargy alternating with irritability and severe axial hypotonia with increased muscle tone in the four limbs. Cranial tomography showed bilateral and symmetric basal ganglia hypointensity. Brain MRI demonstrated bilateral and symmetric T2 signal hyperintensity in the globus pallidus, putamen, cerebral peduncles, medulla oblongata and cervical spinal cord. Swelling and restricted diffusion of the affected basal ganglia, together with a prominent lactate peak in the magnetic resonance spectroscopy of the left basal ganglia, suggested acute damage caused by Leigh syndrome (Fig. S1). The patient became less responsive and more hypotonic, despite treatment with biotin and thiamine, and developed an abnormal respiratory pattern leading to progressive respiratory failure requiring ventilator support. She presented with brief generalized seizures that responded well to diazepam, but the progression of symptoms led to her death 5 days after admission.

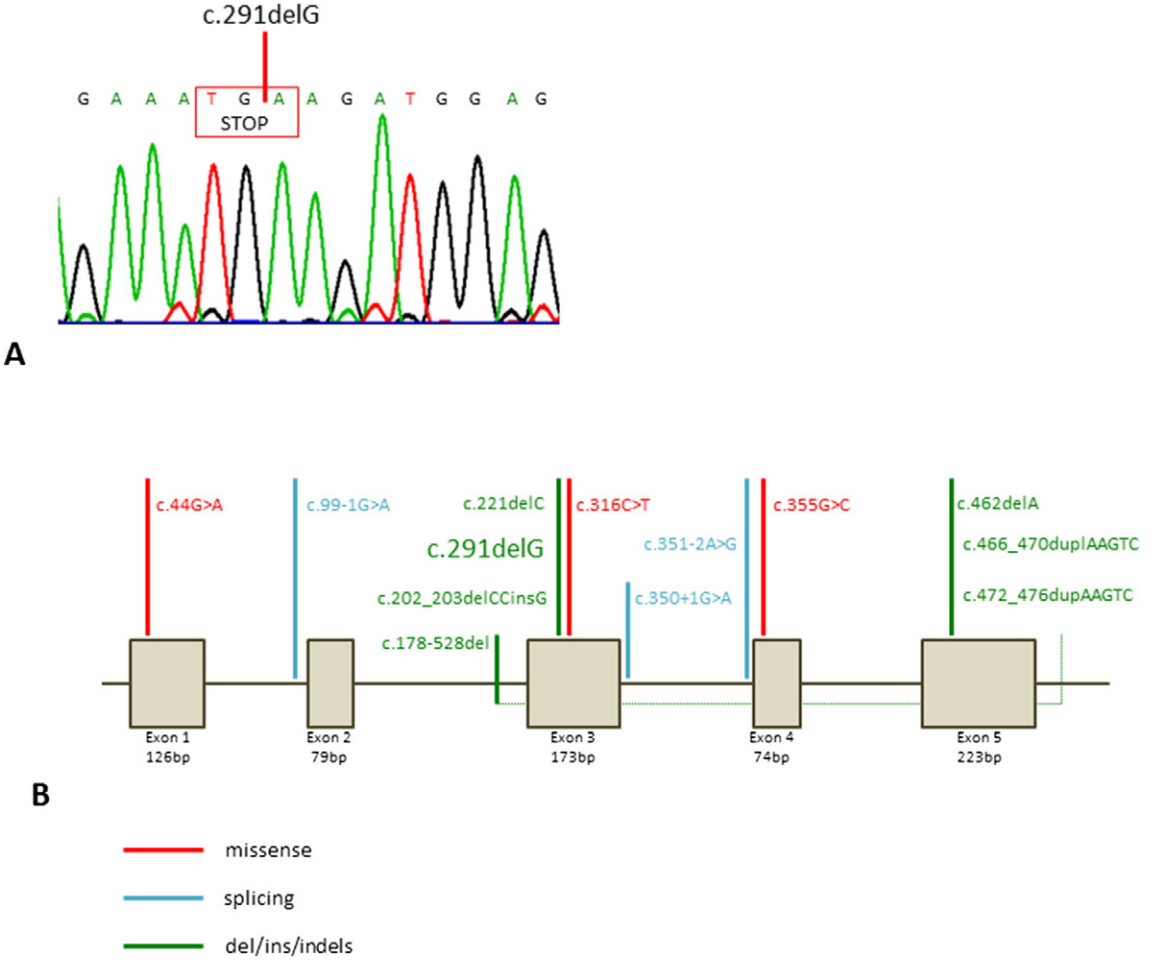

Fig. 1. (A) Sanger confirmation of the homozygous mutation, c.291delG (p.Trp97Ter), in the *NDUFS4* gene and (B) all human *NDUFS4* (NM_002495.2) described mutations.